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**13. ABSTRACT** *(Maximum 200 words)*

Ductal carcinoma *in situ* (DCIS) is a pre-invasive stage of breast cancer in which the malignant cells have not penetrated the basement membrane. From our initial allelotyping study of DCIS we determined that the background rate of loss of heterozygosity (LOH) is low (5%). The chromosomal regions showing LOH significantly above background were 8p, 13q, 16q, 17p and 17q. In the previous report we described the LOH mapping of a regon on 8p (30%LOH) containing a putative tumor suppressor locus to a small interval of ~1.4 cM between the markers D8S265 to D8SD520. During the past year we have continued analysis of this region with the goal of cloning and characterizing a putative breast cancer gene. We have constructed radiation hybrid maps which indicate that the physical distance of this region is ~510 to 850 Kb in length. We assembled a YAC contig covering the region D8S550 through WI-8953 and collected YACs for D8S520. We are currently completing a YAC/BAC contig for the interval between D8S520 and D8S550 which will result in a single contig for the critical region containing the putative tumor suppressor locus.

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**Annual Report for Grant Number DAMD17-94-J-4293, October 1996-September 1997.**

**Tumor Suppressor Genes in Early Breast Cancer and its Progression**

**Helen Donis-Keller, Ph.D.**

**Table of Contents**

## Introduction

### A. Response to Reviewer of 1996 Progress Report

The comments and recommendations made by the reviewer of this proposal were very much appreciated. The comment that "accomplishment of Task 4, cloning of a tumor suppressor locus would require all of the resources of the project" and the reccommendation that "the PI might consider a change in the Statement of Work to reflect the apparent focus on Task 4" have been taken under advisement and I have changed the focus of the project to Task 4 accordingly.

At the same time the reviewer expressed some concern that there were not more tumors that verified the small interval (1.4 cM) containing a putative tumor suppressor locus. While I agree that it would be nice to have hundreds more tumors examined, previous results in my laboratory and the work of others has repeatedly demonstrated the utility of one single translocation or tumor with a deletion in successfully guiding the search to a narrow region of the genome. We have verified the extent of loss within the critical tumor's DNA using other markers and are proceeding to the next step of the project without delay.

I take issue with the assumption of the reviewer that the project suffered a significant set-back with the departure of Dr. Radford and that there had been a lack of significant progress during the interval between years 1 and 2 of the project under Dr. Radford's leadership. Dr. Radford maintained the position of project leader through September of 1996 and prepared the progress report which was submitted and approved. During that period she oversaw the completion of the laboratory studies which she later described in a full length manuscript that has now been submitted. That work describes a considerable amount of LOH analysis on chromosome 8p using 66 cases of DCIS. In order to complete this study during the project year at issue, 46 new dissections and DNA preparations were required and a new fine structure linkage map was needed to determine the order and spacing of markers used for LOH and to identify possible new ones to be used to narrow the interval. The map construction involved marker optimization and genotyping of 23 linkage markers on a total of 16 CEPH families (only 8 families had been typed by Genethon and this was done by Genethon for only some of the markers). Linkage maps were then constructed (with odds of 1000:1 for placement), errors detected, regenotypings done, and new maps constructed. For the information of the reviewer who questioned how much of the map construction work was actually done under the direction of Dr. Radford, I can assure you that all of the genotyping, error detection, regenotyping and additional error checking was done by Dr. Radford and her technician. My group contributed expertise and trained Dr. Radford's technician so that he could run the computer mapping programs (CRIMAP) himself using our SUN workstation. We were available for computer assistance and for help with error detection and checking. The work performed by Dr. Radford and her staff member was indeed significant and useful. There were discrepancies of order between maps previously reported by several groups for some of the markers, particularly in a key region for LOH and, in addition, not all of the markers were included in a single reference map. Dr. Radford's mapping efforts provided a more definitive linkage map for this region and contributed additional genotypic data that can be used by others for future 8p map analyses.

Likewise I take issue with the formally stated lack of confidence that the reviewer seemed to have with regard to a smooth transition of the project to my laboratory that Dr. Radford arranged prior to her departure from Washington University to join a private practice. The project changed hands within a matter of days and the work has continued essentially without interruption. As the progress report for this reporting period shows, we have made significant advances on the cloning of a putative tumor suppressor gene on chromosome 8p.

I appreciate the concern by the reviewer that the positional cloning project was not justfied since the primary data showing the LOH for the critical tumor were not included as figures in the progress report and because the reviewer thought that the 1.4 cM might translate to a physical distance of 2-3 Mb, which "is not an ideal length for positional cloning." I agree with the reviewer, particularly given the limited resources available for this project that a 2-3 Mb region would pose a potentially lengthy project, even given the rapid progress by the large mapping and sequencing centers who make clones and data available to the scientific community. Fortunately, we have now completed a radiation hybrid mapping of the region from which a physical distance has been calculated to be in the range of ~500 - 850 Kb, which should allay the concerns of the reviewer. For the information of the reviewer, Dr. Radford and I have been very conservative in the scoring of loss versus retained alleles as previous figures in our papers have demonstrated, and as did the example shown in figure 2 included in the previous progress report. The submitted manuscript for the 8p mapping shows the gels for the critical tumor in the region of loss and the nearby retained alleles (i.e. markers 550 and 265).

Regarding the reviewer's request for clarification of the term "informative/non-informative" (pg. 6 of 1996 report cited by the reviewer), informative means the number tumor/normal pairs that demonstrated two alleles for the marker in the normal tissue sample, whereas non-informative means that the patient DNA was homozygous for the marker alleles in the normal tissue sample, i.e. therefore loss of an allele, or loss of heterozygosity (LOH) in tumor DNA would not be detectable with the gel assay if the patient DNA was homozygous for a given marker.

Regarding the reviewer's query on why we did not focus on chromosome 17p instead of 8p for gene cloning (reviewer cites as data 18.7% LOH for 8p region whereas 37.5% for 17p). As we have continued to perform LOH analysis on 8p we have found 30% LOH for 8p with the set of DCIS samples as described in our previous progress report, which to us justifies further investigation. In addition, there are a number of research groups focusing on genes contained within 17p that are involved in breast cancer therefore we thought that 17p would be well represented by the work of others.

## B. Nature of the problem

An increasing percentage of breast cancer is being detected at a pre-invasive stage: ductal carcinoma *in situ* (DCIS). DCIS is a form of breast cancer in which malignant cells have not penetrated the basement membrane (1). The histopathological diagnosis encompasses a heterogeneous group of subtypes, including comedo, cribriform, solid, papillary and micropapillary, some of which may differ in biological behavior. The potential for associated micro invasion and likelihood of recurrence after breast conservation therapy are higher with the comedo subtype (2, 3). As with invasive breast cancer, DCIS can also be stratified by nuclear grade: high, intermediate, and low. Circumstantial evidence that DCIS is a precursor lesion to invasive ductal carcinoma is based on three observations: the frequent co-existence of DCIS and invasive cancer in the same breast (4); the greatly increased risk of subsequent invasive breast cancer in women with biopsy-proven DCIS (5); and the finding that when a local recurrence is seen after breast-conserving treatment of DCIS there is a 50% chance that the recurrence will be of the invasive variety (6). DCIS is not an obligate precursor however, and other possible pathways to invasion may exist such as the de novo transition to malignancy of normal epithelium without an intervening non-invasive stage. For many years the standard treatment for DCIS has been total mastectomy, though lumpectomy with adjuvant radiation is being utilized currently for small, well localized areas of DCIS.

Lobular carcinoma *in situ* (LCIS), on the other hand, is not thought to be a pre-invasive cancer but rather an indicator of increased risk of breast cancer. Interestingly, the risk is the same in both breasts regardless of the side in which the LCIS was detected. That the LCIS

cells do not inevitably progress to invasive breast cancer is evidenced by the fact that, of those cancers which do develop, half are of the invasive ductal variety (7).

Atypical lobular hyperplasia (ALH) and atypical ductal hyperplasia (ADH) are considered to be high-risk lesions both associated with an increase of 4-5 fold compared to the general female population. If a strong family history of breast cancer exists, the risk is doubled to 8 to 9 fold (5).

Our studies have concentrated on the genetic changes which occur in DCIS and the transition from DCIS to invasive breast cancer. A better understanding of the oncogenesis of breast cancer at the molecular level, and the correlation of this information with clinical data, may aid in treatment choices.

## C. Background of Previous Work

Most solid tumors arise due to the inactivation of tumor suppressor genes and activation of oncogenes. The accumulation of genetic changes is believed to result in the invasive followed by the metastatic phenotypes. Loss of heterozygosity (LOH) of one of a pair of alleles in tumor tissue compared to matched normal control can reveal areas of chromosome deletion which are likely to contain putative tumor suppressor genes. A large number of articles have been published on LOH in invasive breast cancer, and virtually every human chromosome has been shown to exhibit allelic loss (8,9). The most frequent losses in invasive breast cancer are seen on chromosome 7q (0 -83%) (8,10,11), 16q (32-63%) (8,9,12-14), 17p (31-75%) (8,9,15-18), 17q (24-79%) (8,9,19-25), and 18q (24-69%) (8,22,26,27). Less frequent losses are found on 1p (3-47%) (8,9,28), 1q (16-32%) (8,9,29,30), 3p (11-47%) (8,9,17), 6q (9-48%) (8,9), 8p (27-33%) (8,16) 11p (10-41%) (8,31) and 13q (16-40%) (9,17).

Several investigators have reported two distinct regions of loss on 8p in breast cancer, located at 8p21 and 8p22. Yaremko et. al. studied 20 examples of invasive ductal cancer and found the overall rate of LOH on 8p to be 55% with loss at 8p22 observed more frequently than at 8p21 (32). On the other hand, Aldaz et. al. found loss on 8p in only one of 15 informative samples of DCIS (7%) (33). At the time of our progress report last year we had assayed for LOH using 8 markers on 8p. Of 55 informative samples, LOH was found for at least one 8p marker in 15 tumors (27.3%) (34).

Because of the multiple putative tumor suppressor loci which exhibit LOH in invasive breast cancer, it is not clear which loci are involved in oncogenesis and which are lost randomly due to the instability conferred by the malignant state of the genome.

Allelotyping involves the comprehensive screen of the genome for LOH in a particular cancer. Generally an initial screen will involve assay with at least one marker from each non-acrocentric chromosomal arm. Thereby the average or baseline level of LOH can be determined. This may vary from 5 to 20% depending on the type of cancer. A significant level of LOH, indicating the site of possible tumor suppressor genes involved in oncogenesis, can be ascertained once the background level is known. Regions which show significant LOH can then be analyzed with additional markers to refine the smallest deleted region which may contain the tumor suppressor gene. The analysis of tumors with a number of markers also permits calculation of the fractional allelic loss (FAL) for each tumor. This has been defined as the total number of chromosomal arms which show LOH divided by the total number of informative arms for that tumor (35,36). FAL has been correlated with patient outcome in colon cancer (35), and may correlate with clinical information in other tumor types.

Fewer reports exist on the molecular changes in DCIS than can be found pertaining to invasive breast cancer. Davidoff et. al. (37) studied 6 examples of synchronous DCIS and invasive cancer for expression of p53 and found the same levels of protein expression in each tissue type. Expression of the oncogenes c-erbB-2 and c-myc is also consistent between coexisting pre-invasive and invasive breast cancer (38,39). Zhuang et. al. studied allelic loss for two loci on 11q13 (INT2 and PYGM). They found that for every case of

DCIS which showed LOH (N=15), loss of the same allele was seen in the corresponding invasive tumor (40). O'Connell et. al. (41) studied four loci [TPO (2pter), D4S192 (4q25-34), D16S265 (16q21) and D17S579 (17q21)] and found that 8 of 10 cases of DCIS shared LOH patterns with more advanced lesions for at least one of the 4 loci.

During the first year of this project we completed the allelotyping of DCIS. Ours was the first laboratory to allelotype DCIS. Our findings were as follows: A total of 61 samples of DCIS were assayed. The average number of informative tumors examined for each marker was 19 (range 8 to 48). The median fractional allelic loss (FAL) was 0.037. The highest % of LOH was shown for loci on 8p (18.7%), 13q (18%), 16q (28.6%), 17p (37.5%) and 17q (15.9%). LOH on 18q was found in 10.7% of informative tumors. FAL was associated with LOH on 17p, with high nuclear grade and with the comedo subtype of DCIS. LOH on 17p correlated with LOH on 17q and on 13q. Additional markers were employed for 16q and 17p to determine the smallest common region of deletion and maps of 17p and 16q were generated (42-44). Aldaz et. al. also studied allelic loss in a total of 23 examples of DCIS. they found the most frequent sites of loss to be on chromosomes 7p, 16q, 17p and 17q (33).

To study genetic changes and the evolution of breast cancer we have assayed for loss of heterozygosity (LOH) in twelve sets of synchronous carcinoma *in situ* (CIS) and invasive cancer, compared to normal control DNA. Microsatellite markers were used which map to each non-acrocentric autosomal arm. Eight tumor sets demonstrated LOH of the same allele in both concurrent invasive cancer and DCIS, for a total of eighteen chromosomal loci. Three of nine tumor sets showed LOH on 11p. In two of these sets LOH was seen on 11p only in the invasive tumor, not the corresponding CIS. One of these tumors also exhibited allelic loss in the invasive tumor for 4 loci, all of which were retained in the non-invasive tumor. For two tumor sets LOH was mirrored in matched DCIS, invasive tumor and lymph node metastasis. The maintenance of LOH for certain loci throughout the stages of breast cancer suggests clonality of the cancer cells. Tumor suppressor loci on 11p may be involved in the invasive phenotype (45).

During the second year of the project we have concentrated our efforts on the refinement of the area of loss on 8p. Simultaneously with that study we have generated a fine structure linkage map of 8p. Genetic mapping efforts indicated that the deleted region (between markers D8S520 and D8S265) spanned an interval of 1.4 cM. During the current project year we have focused on the construction of physical maps for the region using radiation hybrid mapping and clone contig construction methods.

## D. Purpose of the Present Work

Revised Statement of Work:

We have accomplished Task 1, The identification and characterization of the extent of chromosomal deletions in DCIS (Months 1-12). We now plan to make use of the information obtained from Task 1 and focus on Task 4: Cloning a tumor suppressor gene involved in breast cancer (Months 24-48). As described in the previous progress report this effort involves construction of a radiation hybrid map of the region and a second type of physical map, a clone contig to be developed from YAC, BAC and P1 clones. We will then proceed to the identification and characterization of candidate genes that lie within the critical region containing the putative tumor suppressor gene for breast cancer.

Task 2, The study of chromosomal deletions in hyperproliferative breast conditions. (Months 12-24) and Task 3, The study of chromosomal deletions in the progression of DCIS to invasive and metastatic phenotypes (Months 1-36) will be taken up after Task 4 has been accomplished, time and effort permitting.

## E. Methods of Approach

a) Accumulation of specimens.

Collaborations have been established with pathologists in St. Louis area hospitals. Archival paraffin embedded material is collected from several hospitals in St. Louis (Barnes-Jewish, Deaconess, St. Louis University, St. Luke's Hospital and the Outpatient Surgery Center). Either matched archival normal lymph node DNA or leukocyte DNA is used as control. When it is necessary to draw blood for normal control, informed consent is obtained following Institutional Review Board approval. A total of 89 examples of DCIS have been accumulated and assayed for LOH with various markers.

b) Microdissection.

For LOH analysis it is necessary to have a relatively pure tumor sample with little if any contaminating normal stroma. We have been using a microdissection technique to enrich for tumor cells in which an unstained 20 micron thick section from a particular block is overlaid on a stained 5 micron thick section. Landmarks such as blood vessels are aligned and the tumor dissected from the unstained section using a scalpel blade.

c) DNA extraction and LOH analysis.

Following separation of tumor and normal tissue DNA is extracted by digestion with proteinase K, purified with phenol/ chloroform and precipitated with alcohol. DNA is quantified with a fluorimeter. For assay of LOH we have used a panel of highly polymorphic microsatellite markers. Polymerase chain reaction (PCR) is performed in the tumor/normal pairs and the products separated on acrylamide denaturing gels. Reactions have been optimized for 5 to 10 ng of template DNA in order to maximize the number of reactions possible with each tumor. On autoradiography, absence or greatly reduced intensity of one allele in the tumor compared to the heterozygous normal control indicates LOH.

Several samples contain insufficient tumor cells to permit the extraction technique described above. New methods have been developed in order to consistently amplify via PCR these low quantities of DNA. Following microdissection the tumor tissue is digested in a small volume (10-20 microliters) of lysis buffer containing proteinase K. After complete digestion has been determined, samples are phenol extracted once to destroy the proteinase K and chloroform extracted once to remove the phenol. Aliquots of this material are then used directly as a template for PCR amplification. A disadvantage of this method is that only a limited number (10-20) of reactions can be done, and therefore this technique would not be suitable for an allelotyping study.

d) Generation of genetic linkage maps.

Once a region of chromosomal deletion has been identified it can be narrowed down using a panel of closely linked markers which map to that area. Since new microsatellite markers are becoming available daily, they often do not appear on currently published maps. In order to determine the deletion map in the tumors, it is necessary to know the precise location of the markers being used. A fine structure map can be generated using genotypic data from a number of families made available through the Centre d'Etude Polymorphisme Humaine (CEPH). Having identified a small region of deletion (preferably no larger than 1cM) positional cloning techniques can be undertaken to clone the putative tumor suppressor gene contained within the region.

e) Radiation hybrid maps

G3 and GB4 are the two radiation hybrid mapping panels (Research Genetics, Inc. in Huntsville, Alabama) we used to construct the RH placement map. The G3 panel, comprised of 83 RH clones from the whole human genome, was created at the Stanford Human Genome Center and is considered a medium resolution panel (i.e. 500 Kb resolution). The GB4 panel, comprised of 93 RH clones from the whole human genome, has lower resolution (i.e. 1000 Kb). It is a subset of the 199 clone panel developed by the laboratories of Peter Goodfellow and Jean Weissenbach.

STS markers are assayed by PCR amplification and sizing on agarose gels stained with EtBr. Each assay is performed twice, i.e. PCR products at the expected size must be observed on each of the duplicate gels in order to be scored as positive. The data generated from the GB4 panel are submitted to the Whitehead Institute Center for Genome Research (WICGR) Mapping Service Center. The program RHMAPPER at the Center is used to analyze all the submitted markers with their high-likelihood framework map and we are then forwarded a placement map with all the submitted markers including LOD score and the distance in cR between two highest-linked markers. The data generated from the G3 panel is submitted to Stanford RH server which subsequently returns the results of analysis with a list of the highest-linked mapped markers, the LOD score of the link, and the distance in cR between the submitted marker and the linked marker on the map. However, it only compares one submitted marker and the highest-linked marker at a time. In order to construct a map of higher resolution which obtainable with the G3 panel, we used the program RHMAPPER version 1.0 from WICGR, the Stanford RH database and our own G3 data for the 6 markers of interest. We used the RH database from Stanford to build a G3 panel placement map (framework), then we integrated our RH data from the six markers covering the deletion region with the framework map.

f) YAC, BAC, P1 clone contig construction

YACs available from the CEPH library that we maintain in our laboratory were streaked on YPD plates, and 10 colonies from each clone were tested by a "whole cell PCR" assay using the STS markers to identify the positive clones. For "whole cell PCR, a small amount of cells from an isolated colony are suspended in 5 ul of deionized water and the suspension is used directly as the template in a standard PCR reaction. The presence of other markers within the YACs are also assayed by PCR assays of STS markers. The standard ligation-mediated PCR method was used to develop new STSs from YACs.

We screened a BAC library (Research Genetics, Inc., Huntsville, AL) to identify BAC clones for contig construction. STS assays for relevant markers were tested using the 120 standard PCR screening reactions to survey the STS content of the entire library. As with YAC clone isolation, each identified BAC clone was then verified by "whole cell PCR" assay using 10 randomly selected colonies as candidates. After the positive BAC clones were verified, single BAC colonies were propagated in liquid medium, cells harvested and insert DNA prepared using the Plasmid Midi-Kit from Qiagen Inc. (Chatsworth, CA ). Each BAC clone was partially sequenced from the insert ends using T7 and Sp6 primers. The sequence generated from ABI sequencing was analyzed for candidate PCR primer sequences using the program PRIMER 0.5 (Lincoln and Lander, MIT Center for Genome Research). STS assays were developed and then used as new entry points for chromosome walking.

**Body: Experimental Methods Used and Results Obtained.**

**Task 4: Cloning a tumor suppressor gene involved in breast cancer. Months 24-48.**

## RH mapping

In order to efficiently clone and characterize a putative tumor suppressor gene involved in breast cancer we have constructed a radiation hybrid map for the 1.4 cM deletion interval between markers D8S520 and D8S265 within chromosome 8p22-23. This map would provide an independent means of ordering the markers in this region and verify the linkage map order, a necessary step prior to the contruction of a clone contig and gene identification. The radiation hybrid map will also identify additional markers that can be used to construct a clone contig for the region.

Six microsatellite markers, D8S265, D8S520, D8S550, D8S1695, D8S1755 and D8S1759 were used to screen the Stanford G3 and Genebridge 4 RH panels. Each marker was typed twice for each panel. Data from the Stanford G3 panel and the program RHMAPPER version 1.0 (WICGR) were used to construct an RH placement map (Fig. 1). This map spans a distance of 17.0 cR, or approximately 510 - 850 Kb (30-50 kb per cR), with the marker D8S520 placed distal to the centromere and D8S1759 proximal to the centromere (Fig.1). Our typing data using the GB4 panel were submitted to the WICGR Mapping Service Center and the order of these six markers was returned to us. The locations of D8S520, D8S550 and D8S1759 were confirmed, however the position of D8S265, D8S1695 and D8S1755 differ from the position found on the G3 RH placement map. Since the G3 panel is a higher resolution mapping panel (i.e. with more breaks) we expect that the order determined from these data is more reliable than the GB4 result.

## Construction of an integrated YAC/BAC contig

Based on the published Whitehead and CEPH YAC Contig maps, we isolated thirteen YACs using as probes the six markers listed above and two additional markers, WI-6800 and WI-8953 fromWICGR that appeared to fall within the relevant interval. We confirmed the presence of these markers within the YAC clones and verified that three expressed sequence tags (ESTs) reported to lie within this region were contained within the YAC contig (Fig.2). This preliminary physical map confirms the marker order we determined using RH mapping on Stanford G3 panel. We did not identify any YACs that would fill the interval between markers D8S520 and D8S550, therefore one gap existed within the physical map of the region.

Our first approach in building a refined physical map is to close the gap between YACs 700D3 and 770E9 anchored by D8S520 and D8S550. This involved screening a human Bacterial Artificial Chromosome (BAC) library and determining insert end sequences from which additional PCR assays could be developed and used to identify additional BACs and YACs for contig construction, i.e. chromosome walking. Thus far we have identified and partially characterized 16 BAC clones (Fig. 3). From the initial screening of the BAC library, five clones were identified and isolated using a PCR assay from the marker D8S520. From the BAC clone A1 a PCR assay A1t was developed and mapped to BAC clones A3, A4, A5 and the YAC 700D3, thus solidifying the contig. Similarly, insert end assays (C2t, C2s) were developed from both ends of the BAC clone C2. Both assays tested positive for BAC clones C1 and C3 and the YAC clones 770E9 and 723F10. In addition, a novel STS (770PLL) was developed from YAC 770E9 using the ligation-mediated PCR method. This assay mapped back to the YAC contig and rodent/human hybrid panel mapping of this assay provided verification that the end of this clone mapped to chromosome 8. The 770PLL assay also identified 6 BAC clones that will be useful source material for generation of additional end-clone assays for additional chromosome walking. The development of new STSs from these clones is in progress.

The EST A mapped to YACs 770E9, 915H4, 729E12, 715C10, and 737E5. This EST appears to be a unique sequence since it did not identify any homologous sequences in the available databases. EST B mapped to YAC 809H8 and is one of the ESTs identified

by the marker SGC30677 (derived from a cDNA clone similar to human Farnesyl-Diphosphate Farnesyltranferase). EST C localized to the same YAC as EST B. It is derived from the marker A005M25 which is an apparently unique transcript.

## Conclusions

Our radiation hybrid mapping efforts conducted during this project year have independently determined the order of genetic markers for the smallest region of deletion found during the previous project year (i.e. 1.4 cM within 8p22). The physical distance spanned by the critical markers appears to be 17 cR or approximately 510 Kb - 850 Kb, a region that most scientists would judge feasible for positional cloning. The RH map also identified additional markers within the interval from which a clone contig construction project was initiated. YACs were identified from this region and one gap (between markers D8S520 and D8S550) was identified. Through a combination of YAC and BAC screening, STS development from end clone sequences, and rescreening the YAC and BAC libraries, we are proceeding with closing the gap between these critical markers. We will also implement P1 screening as necessary in order to complete the contig construction. Future plans will involve gene identification and characterization in an effort to identify a tumor suppressor gene that is implicated in the development of DCIS.

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## Appendices

Figure 1. G3 RH placement map constructed by typing 6 markers within the deletion region.

Figure 2. YAC contig on Ch. 8p 22-23 covering the 1.4 cM deletion region.

Figure 3. Integrated YAC/BAC contig between the markers of D8S520 and D8S550.

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| Marker | Distance (cR) | Type |
|---|---|---|
| Telomere | | |
| **D8S520** | **0.0** | **P>3.00** |
| SHGC-1941 | 6.2 | F |
| SHGC-1962 | 1.5 | F |
| **D8S550** | **3.5** | **P>3.00** |
| **D8S1755** | **1.4** | **P>3.00** |
| **D8S265** | **0.0** | **P0.00** |
| SHGC-3114 | 2.9 | F |
| SHGC-18016 | 1.5 | F |
| **D8S1695** | **0.0** | **P>3.00** |
| **D8S1759** | | **P>3.00** |
| Centromere | | |

Figure 1. G3 RH placement map constructed
from the typing of six markers within the deletion region.
P: retention probability; F: frame work

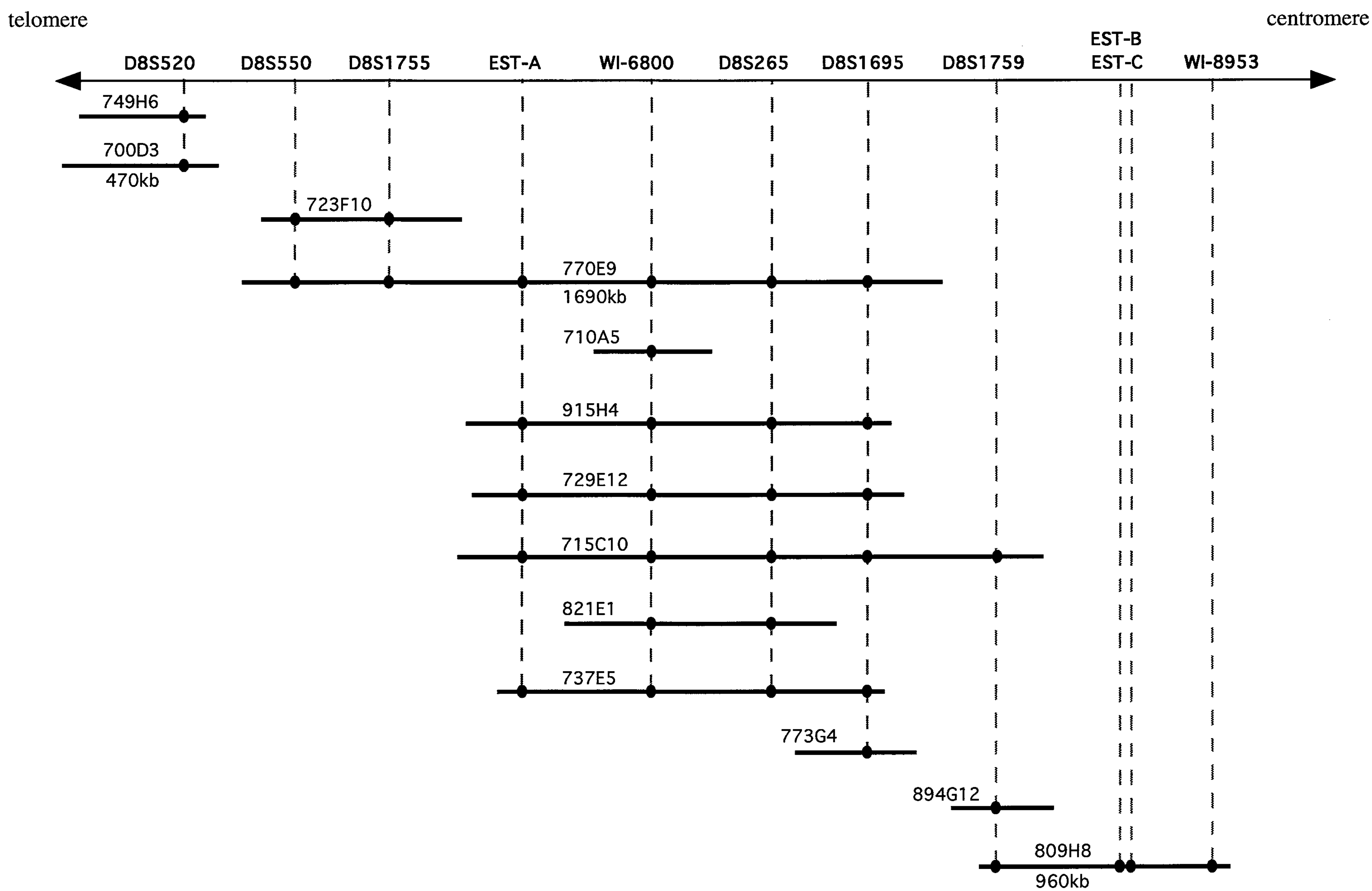
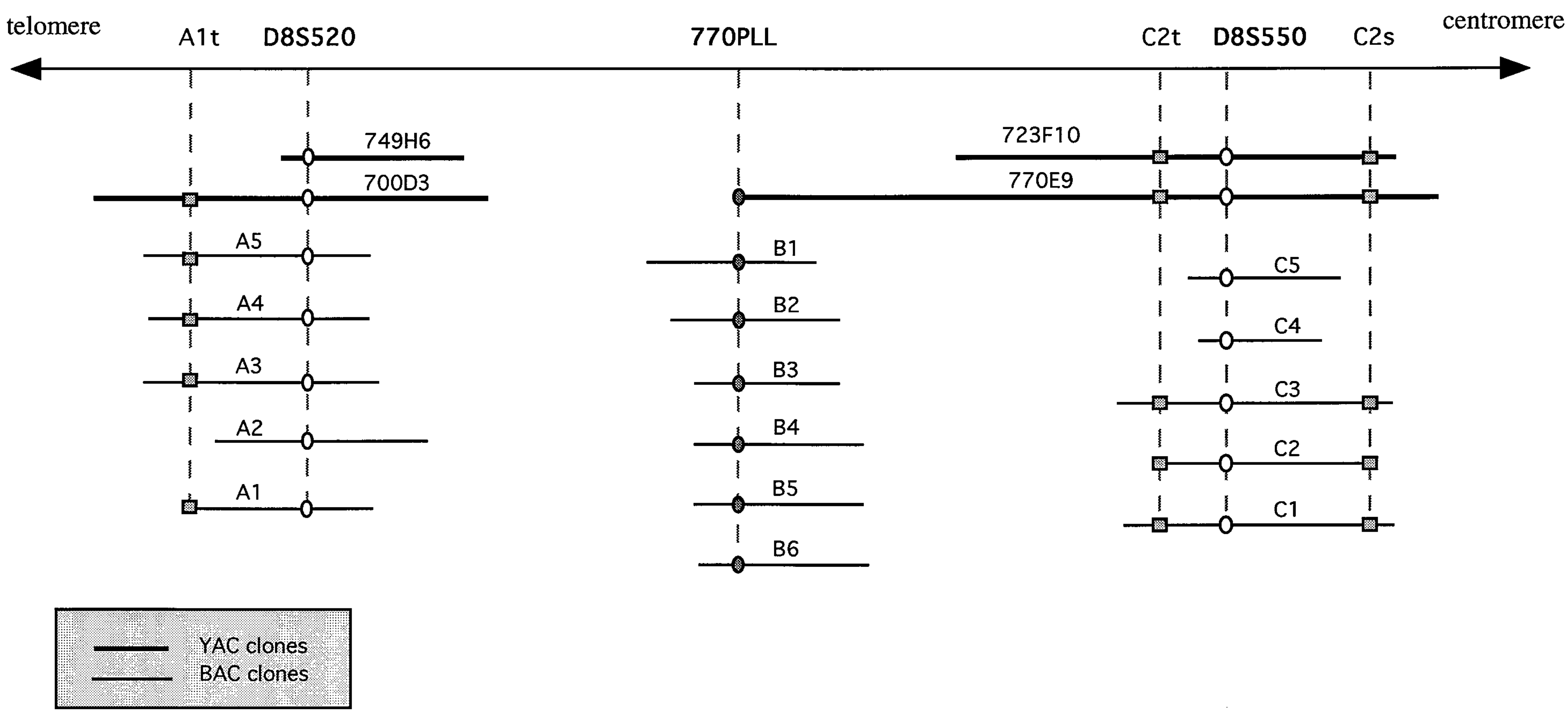



Figure 2. YAC contig on Ch.8p 22-23 covering the 1.4 cM deletion region.

Figure 3. Integrated YAC/BAC contig between the markers of D8S520 and D8S550.



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**Abstract title:** Chromosome 8p deletions in ductal carcinoma \italicize{in situ} (DCIS) of the breast.

**Abstract author(s):** J. C. Wang, D. Radford, M. Holt, C. Helms, W. Brandt, and H. Donis-Keller.

**Abstract Institution(s):** Div. Hum. Mol. Genet., Dept. Surgery, Washington University School of Medicine, St. Louis, MO.

**Abstract body:** In order to efficiently clone and characterize a putative tumor suppressor gene for DCIS that we have mapped within chromosome 8p (Radford et al., 1995, Can. Res. 55:3399), we are constructing a fine structure integrated physical and genetic map for this region. A linkage map was first constructed using 23 markers we genotyped through the CEPH reference pedigrees with loci uniquely placed (odds for order 1000:1). The map extends from the telomere of 8p to just beyond the centromere (80.8 cM). The average distance between markers is 3.5 cM. \parBased on this map, 18 markers on 8p and two 8q markers were selected for LOH analysis with 65 examples of microdissected DCIS. Of 61 informative specimens, LOH was observed for at least one marker on 8p in 17 tumors (28\%). Ten tumors have lost a large portion of the short

arm. The smallest common region of deletion localizes to a region of 1.4 cM on 8p22-23. To refine this region, six markers were used to screen Stanford G3 and Genebridge 4 RH panels. The data from the Stanford G3 panel were analyzed and a RH map has been constructed using RHMAPPER. The map spans 17.1 cR in distance with the marker D8S520 placed distal to the centromere and D8S1759 proximal to the centromere. The data from the GB4 panel were submitted to the WICGR Mapping Service center and the order of these six markers has been determined. The locations of D8S520 and D8S1759 were confirmed, however the position of D8S265, D8S1755, and D8S1695 differ from the position found on the G3 RH map. Currently we are using 7 markers and 4 ESTs to screen YAC and BAC libraries for the construction of a contig for this region. A preliminary physical map based on clones isolated thus far confirms the marker order we determined using the Stanford G3 RH panel.

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Rec'd 7/23/2001

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